

DP Barcode: 358148

MRID No.: 47560131

DATA EVALUATION RECORD HONEY BEE - ACUTE CONTACT & ORAL LC₅₀ TEST OECD TG 214 (OPPTS 850.3020) & OECD TG 213

1. **CHEMICAL**: DPX-MAT28 Technical (Aminocyclopyrachlor acid)

PC Code No.: None

2. **TEST MATERIAL**: Aminocyclopyrachlor acid

Purity: 92.2%

3. **CITATION**

Authors: Warmers, C.

Title: DPX-MAT28 Technical: Acute Oral and Contact Toxicity to the Honey Bee, *Apis mellifera* L.

Study Completion Date: November 12, 2007

Laboratory: Eurofins-GAB GmbH, Germany

Sponsor: E.I. du Pont de Nemours and Company, Wilmington, Delaware

Laboratory Report ID: 20071090/01-BLEU

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4. **REVIEWED BY**: Moncie Wright, Staff Scientist, Cambridge Environmental, Inc.

Signature:

Moncie V Wright

Date: 06/02/09

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

Signature:

Teri S Myers

Date: 07/23/09

5. **APPROVED BY**: Anita Ullagaddi, EPS, OPP/EFED/ERB1

Signature:

Anita Ullagaddi

Date: 10/07/09

6. **DISCLAIMER**: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to honey bees via oral and contact exposure routes. It is not intended to prescribe conditions to any external party for conducting this study or to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may



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be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS:

Age of Test Organism at Test Initiation:	Young adult workers (females)
Type of Concentrations:	Nominal (Contact) and Actual Uptake (Oral)
Definitive Test Duration:	48 hours for both tests

8. CONCLUSIONS:

Contact Test

-mortality: negative control – 0%; 6.25-100 µg ai/bee- 0% mortality
-sub-lethal effects- none

Oral Test

-mortality: negative control- 0%; 7.39-112.03 µg ai/bee- 0-2% mortality
-sub-lethal effects- At 24 hours, 1 bee from the 28.09 µg ai/bee test level was apathetic, and 8 bees from the 112.03 µg ai/bee test level had coordination problems. These behaviors were not observed at 48 hours.

Results - Contact Test:

LD ₅₀ : >100 µg ai/bee	95% C.I.: N/A
NOAEL: 100 µg ai/bee	Probit Slope: N/A
LOAEL: >100 µg ai/bee	

Results - Oral Test:

LC ₅₀ : >112.03 µg ai/bee	95% C.I.: N/A
NOAEL: 112.03 µg ai/bee	Probit Slope: N/A
LOAEL: >112.03 µg ai/bee	

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: The acute contact portion of this study satisfies the 850.3020 guideline requirements. The acute oral portion of the study does not have a corresponding OPPTS 850 guideline; however, the results of the study are scientifically sound.

C. Reparability: N/A

10. GUIDELINE DEVIATIONS:

1. Relative humidity in this study was reported as ranging from 48 to 80%; OPPTS guidelines suggest that humidity should be maintained between 50 and 80%.
2. The age of the bees was not reported.
3. Temperature in this study ranged from 23 to 26°C; OPPTS guidelines suggest that temperatures be maintained between 25 and 35°C.

11. SUBMISSION PURPOSE: This study was submitted to provide the effects on honey bees (*Apis mellifera carnica* L.) following acute contact and oral exposure to aminocyclopyrachlor acid for the purpose of new chemical registration.

12. MATERIALS AND METHODS:**A. Test Organisms**

Guideline Criteria	Reported Information
Species: Species of concern (<i>Apis mellifera</i> , <i>Megachile rotundata</i> , or <i>Nomia melanderi</i>)	<i>Apis mellifera carnica</i> L.
Age at beginning of test:	Not reported
Supplier:	Beekeeper in Rheinland-Pfalz, Germany (responsible beekeeper: Mr. Berthold Nengel, Bruckensteinstraße 12, 56348 Dahlheim)
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Stainless steel cages (10 x 8.5 x 5.5 cm; L x W x H) with a removable glass sheet on the front side; inner walls lined with filter paper; bottom perforated with ventilation holes (1 mm diameter)
Lighting:	Constant darkness (except during observations)
Temperature:	23-26°C
Relative humidity:	48-80%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported.
Reference toxicant test?	Perfekthion (active ingredient dimethoate; 414.4 g/L purity)
Method of administration:	<u>Oral test:</u> Dissolved in water, then diluted with a 50% aqueous sucrose solution <u>Contact test:</u> Dissolved in tap water; after anesthetization with CO ₂ , 2 µL applied to dorsal thorax
Nominal doses:	<u>Contact and oral test:</u> 0 (negative control), 6.25, 12.5, 25, 50, and 100 µg ai/bee
Controls: Negative control and/or diluent/solvent control	<u>Oral test:</u> negative control – 50% sucrose solution <u>Contact test:</u> negative control – tap water with anesthetization
Number of colonies per group:	<u>Oral test:</u> 5 reps, 10 bees per rep <u>Contact test:</u> 5 reps, 10 bees per rep
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	<u>Oral test:</u> N/A <u>Contact test:</u> N/A
Feeding:	50% aqueous sucrose solutions, <i>ad libitum</i>
Observations period:	4, 24 and 48 hours after application

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. The Principles of Good Laboratory Practice, Chemical Act, Attachment 1, Germany (2002); and OECD Principles of GLP (ENV/MC/CHEM(98)17; 1998), which are both consistent with the requirements of the U.S. EPA, FIFRA, Title 40 CFR Part 160 (1989) the Japan Ministry of Agriculture, Forestry, and Fisheries, 59 NohSan, Notification No. 3850 (1984).
Control performance:	<u>Oral test</u> : 100% survival <u>Contact test</u> : 100% survival
Raw data included:	Yes
Signs of toxicity (if any) were described?	Yes

Mortality - Oral Test

Dosage µg ai/bee (actual intake)	No. of Bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance			
Negative Control	50	0	0
7.39	50	2	2
13.84	50	0	0
28.09	50	0	0
53.86	50	0	0
112.03	50	0	0
Toxic Standard (µg ai/bee -actual intake)			
0.09	50	20	26
0.10	50	40.8	44.9
0.14	50	78	82
0.21	50	98	98

Observations:

At 24 hours, 1 bee from the 28.09 $\mu\text{g ai/bee}$ test level was apathetic, and 8 bees from the 112.03 $\mu\text{g ai/bee}$ test level had coordination problems. These behaviors were not observed at 48 hours. Mortality was 0% in the 112.03 $\mu\text{g ai/bee}$ treatment group, which was the highest test level. The resulting LC_{50} value was $>112.03 \mu\text{g ai/bee}$.

Mortality - Contact Test

Dosage µg ai/bee	No. of Bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance			
Negative Control	50	0	0
6.25	50	0	0
12.5	50	0	0
25	50	0	0
50	50	0	0
100	50	0	0
Toxic Standard (µg ai/bee)			
0.10	50	6	10
0.14	50	20	48
0.22	50	30	40
0.30	50	96	96

Observations: No sub-lethal effects were observed in the negative control or the treatment groups. Mortality was 0% in the 100 µg ai/bee treatment group, which was the highest test level. The resulting LD₅₀ value was >100 µg ai/bee.

Statistical method: Mortality was <50% at each test level, and no differences were observed between the control and treatment groups. Therefore, statistics were not conducted on the cumulative mortality in the oral and contact test.

Reported Statistical Results - Oral Test:

LC₅₀: >112.03 µg ai/bee 95% C.I.: N/A
 NOAEL: 112.03 µg ai/bee Probit Slope: N/A
 LOAEL: Not reported

Reported Statistical Results - Contact Test:

LD ₅₀ : >100 µg ai/bee	95% C.I.: N/A
NOAEL: 100 µg ai/bee	Probit Slope: N/A
LOAEL: Not reported	

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: A lack of mortality precluded statistical analysis. Toxicity values were determined visually.

Results - Oral Test:

LC ₅₀ : >112.03 µg ai/bee	95% C.I.: N/A
NOAEL: 112.03 µg ai/bee	Probit Slope: N/A
LOAEL: >112.03 µg ai/bee	

Results - Contact Test:

LD ₅₀ : >100 µg ai/bee	95% C.I.: N/A
NOAEL: 100 µg ai/bee	Probit Slope: N/A
LOAEL: >100 µg ai/bee	

15. REVIEWER'S COMMENTS:

Relative humidity in this study was reported as ranging from 48 to 80%; OPPTS guidelines suggest that humidity should be maintained between 50 and 80%.

The age of the bees was not reported.

Temperature in this study ranged from 23 to 26°C; OPPTS guidelines suggest that temperatures be maintained between 25 and 35°C.

The study author reported following OECD Guidelines No. 213 and 214 (1998).

16. REFERENCES:

Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1, in der Fassung der Bekanntmachung vom 20. Juni 2002 (BGBl. I S. 2076).

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